Incidence of Aflatoxin in Southern Corn, 1969-1970[1]

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THREE PREVIOUS SURVEYS (1–3) of corn of all grades collected in 1964, 1965, and 1967 from the Midwest revealed low incidences and levels of aflatoxin. Incidences ranged from 1.7 to 2.3%. Levels of aflatoxin ranged from 3 to 27 p.p.b. (total of aflatoxins B-1 and G-1). Most of the samples found to contain toxin were in the poorest grade. A fourth survey (4) was conducted on 293 export corn samples of all grades except U.S. grade No. 1 collected at 10 ports. Eight of these samples contained from 6 to 25 p.p.b. B-1. One sample was in Sample Grade (SG), three in U.S. grade No. 4, three in U.S. No. 3, and one in U.S. No. 2.

Of the 48 aflatoxin-positive samples in the 2,117 corn samples tested in the four surveys mentioned, only four contained more than 20 p.p.b. aflatoxin B-1, the administrative guideline established by the Food and Drug Administration (FDA). The FDA position is that there is inadequate toxicological evidence to establish a tolerance in man. However, in situations where a low level of aflatoxin might be unavoidable in primary agricultural commodities, the FDA has established an administrative guideline consistent with practical accomplishments of current technology and available analytical methodology. The guideline is not applicable to situations that can be avoided, such as blending of contaminated with uncontaminated lots to reduce toxin levels. The guideline can be changed by FDA administrative decisions (5).

The results of the four surveys did not appear to be alarming because of the low incidence and low levels of aflatoxin detected. In two separate instances, however, aflatoxin has been reported in corn from the South. A field study (6) in North Carolina of aflatoxicosis in broiler chickens revealed that the primary source of aflatoxin in the mixed feed was corn. Analyses of corn going into the feed showed a 30% incidence of toxin contamination in the samples. Earlier Wilson et al. (7) assayed seven corn samples associated with toxic hepatitis in swine and cattle in the southern U.S. and found five contained aflatoxin in amounts ranging from 50 to 280 p.p.b. Considering reports of aflatoxin contamination, we decided to examine southern-grown corn from commercial markets. The states from which we got the corn for the study accounted for 4% of the total corn produced in the U.S. during 1969 and 1970.

TABLE I. DISTRIBUTION OF SAMPLES IN SOUTHERN CORN SURVEY FOR AFLATOXIN

Origin	Grade (U.S. No.) 2	3	4	5	SG[a]	Total
Alabama	2	7	5	4	2	20
North Carolina	3	3	3	2	2	13
South Carolina	1	4	2	3	0	10
Tennessee	1	2	1	0	2	6
Virginia	3	3	2	2	1	11
	10	19	13	11	7	60

[a]SG = Sample Grade.

TABLE II. SURVEY OF SOUTHERN CORN (CROP YEARS 1969-1970) FOR AFLATOXIN

Grade (U.S. No.)	Samples Tested	Aflatoxin-Positive Samples	Percent Incidence[a]
2	10	2	20
3	19	6	31
4	13	5	38
5	11	6	55
SG	7	2	29
Total	60	21	

[a]Percent incidence in all grades is 35.

[1]Presented at the 57th Annual Meeting, Miami Beach, October-November, 1972.

[2]Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Materials and Methods

Collection and Preparation of Samples. Samples (1 kg.) of all grades of yellow and white corn were collected from commercial markets in five southern states (Table I) from July 1969 to December 1970. Results apply to both yellow and white corn except where specified. The Grain Division, Agricultural Research Service, collected and graded the samples. Samples were prepared as in earlier surveys (1,2,4).

Extractions and Column Chromatography. The method of extraction and column chromatography appearing in AOAC Official Methods of Analysis was used (8,9). Ground corn was extracted with chloroform and water and extracts were partially purified for thin-layer chromatography on silica gel columns.

Thin-Layer Chromatography. Partially purified preparations from silica gel columns were chromatographed on Adsorbosil-1 thin-layer plates developed with acetone:chloroform:water (12:88:1.5 v./v./v.) (10). The sensitivity limit of the assay as carried out was 1 to 3 p.p.b. aflatoxins B-1 and G-1.

Confirmatory Tests. The identity of aflatoxin B-1 was confirmed by the formation of water and acetate adducts (8).

Examination of Samples for Fungi. The methods used for the microbiological examination of corn samples from the South were the same as those previously used (11).

Results and Discussion

The results of the survey of southern corn are summarized in Table II. Of the 60 samples analyzed, 21 contained aflatoxin. The incidence (35%) was higher than those found in previous surveys of corn collected from other areas in crop years 1964, 1965, and 1967. Positive samples were observed in all grades. The incidence in white corn (36%) was about the same as the incidence in yellow corn (35%), but fewer samples of white corn were examined. No attempt was made to obtain any definite distribution of samples among different grades because so few samples were available during the collection period at these commercial markets. The presence of aflatoxin was confirmed by the formation of water and acetate addition

TABLE III. SOUTHERN CORN SAMPLES CONTAINING AFLATOXIN (SAMPLES ASSAYED = 49 YELLOW AND 11 WHITE CORNS)

Sample	Grade (U.S. No.)	Type of Corn	Aflatoxin Present	Level p.p.b.	*Aspergillus flavus*	Grading Information: Moisture %	Grading Information: Damaged kernels total %	Grading Information: Broken corn and foreign material %	Crop Year	Origin
F-4543	2	Yellow	B-1	62	+[a]	15.3	3.6	2.6	1970	North Carolina
F-4552	2	Yellow	B-1	62	+[b]	15.1	2.6	2.3	1970	North Carolina
			B-2	5						
F-4514	3	Yellow	B-1	13	+	12.2	1.3	3.5	1969	Tennessee
F-4538[c]	3	Yellow	B-1	83	+	17.5	2.5	1.8	1970	South Carolina
			B-2	6						
F-4542	3	Yellow	B-1	12	+	15.7	2.6	3.0	1970	North Carolina
			B-2	2						
			G-1	10						
			G-2	<2						
F-4547	3	Yellow	B-1	25	+	17.5	2.3	2.0	1970	Virginia
			B-2	7						
F-4549	3	Yellow	B-1	6	+	17.4	6.9	1.5	1970	South Carolina
F-4559	3	Yellow	B-1	114	+[b]	9.4	3.3	4.0	1970	South Carolina
			B-2	12						
			G-1	Trace						
F-4504	4	Yellow	B-1	31	+	13.8	2.8	4.4	1969	Alabama
			B-2	3						
F-4513	4	White	B-1	6	+	13.7	1.5	4.4	1969	Tennessee
F-4539	4	White	B-1	12	+	13.1	4.2	4.4	1970	South Carolina
F-4546	4	Yellow	B-1	12	+	18.8	0.9	2.1	1970	North Carolina
			B-2	2						
F-4555	4	Yellow	B-1	6	+	15.5	8.1	5.0	1970	North Carolina
			B-2	Trace						
F-4503	5	Yellow	B-1	308	+[b]	20.5	1.1	5.2	1969	Alabama
			B-2	40						
F-4506	5	White	B-1	141	+	20.6		1.0	1969	Alabama
			B-2	27						
			G-1	6						
			G-2	1						
F-4537	5	White	B-1	42	+[b]	13.0	5.4	5.4	1970	South Carolina
			B-2	8						
F-4554[c]	5	Yellow	B-1	124	+[b]	19.1	12.0	2.0	1970	South Carolina
			B-2	12						
F-4556	5	Yellow	B-1	8	+	15.2	14.1	2.5	1970	North Carolina
			B-2	Trace						
F-4558	5	Yellow	B-1	61	+	14.9	7.6	5.5	1970	Virginia
			B-2	5						
			G-1	Trace						
F-4516[d]	SG	Yellow	B-1	4	+	11.4	17.4	2.5	1969	Tennessee
F-4517	SG	Yellow	B-1	98	+[b]	11.0	16.1	2.6	1969	Tennessee
			B-2	9						
			G-1	Trace						

[a] + = *A. flavus* present.

[b] *A. Flavus* is predominant mold and usually overgrew mold profile plates.

[c] These corn samples were weevily.

[d] This sample was heating 1.3%.

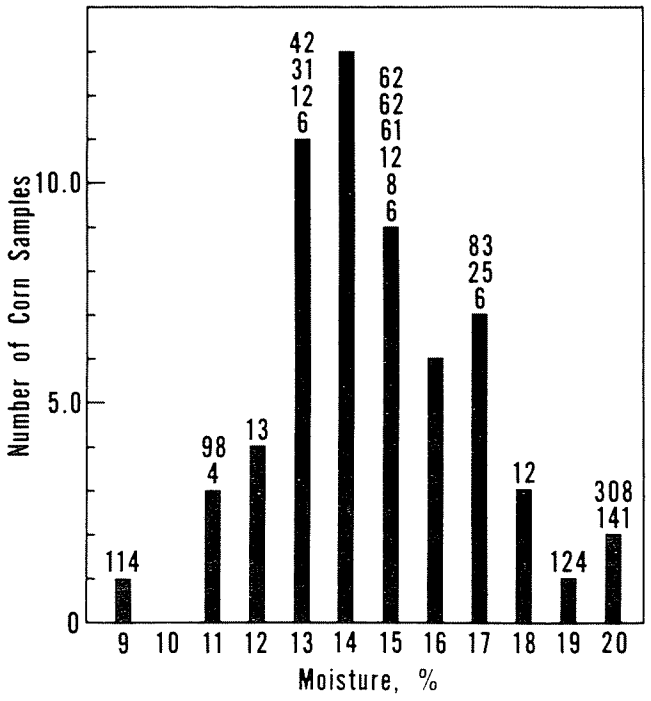

Fig. 1. Moisture content of southern corn samples assayed for the presence of aflatoxin. Numbers indicate levels of aflatoxin (p.p.b.) found in a positive sample with that percent moisture.

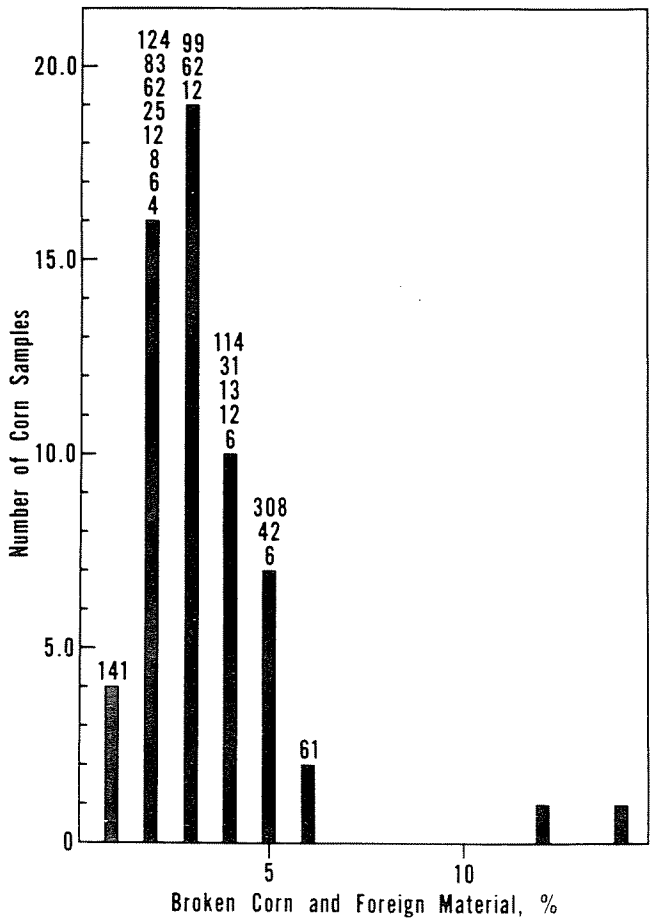

Fig. 2. Percentage of broken corn and foreign material in southern corn samples assayed for the presence of aflatoxin. Numbers indicate levels of aflatoxin (p.p.b.) found in a positive sample with that percent broken corn and foreign material.

compounds for all of the 21 samples reported as positive.

The aflatoxins detected, the levels determined, grading information, and other pertinent information on samples are given in Table III. In previous surveys (1,2,4), very few samples contained appreciable amounts of G-1 along with B-1, and B-2 was not always detected in samples containing B-1. Similar results were obtained in the survey of southern corn. Aflatoxin G-1 was detected in five samples, but only traces were present in three of these. Aflatoxin B-2 was always present in lower concentrations than B-1 and was not even detected in six samples.

All 12 samples of corn analyzed of the 1969 crop from the Southeast (North Carolina, South Carolina, and Virginia) were found to be aflatoxin-negative. Of the 22 corn samples from the 1970 crop assayed, 14 contained aflatoxin. During the 1970 crop year, the Southeast had an epidemic of southern corn leaf blight. It is tempting to postulate that injury by the southern corn leaf blight organism might have left the corn in 1970 more vulnerable to invasion by *Aspergillus flavus* and subsequent toxin formation. However, there is not sufficient evidence to establish a relationship between southern corn leaf blight infection and aflatoxin contamination.

Contaminated corn samples containing more than 20 p.p.b. aflatoxin are the ones that cause concern. Of the 21 southern corn samples from crop years 1969 and 1970 found to contain aflatoxin, 12 samples had toxin levels greater than 20 p.p.b., the guideline (Table III), in contrast to results in earlier surveys (1,2,4) where lower levels were encountered.

Aspergillus flavus was detected in all samples that contained aflatoxin. In six of the 21 positive samples, *A. flavus* was the predominant mold and usually overgrew the plates. In these six samples, the levels of aflatoxin B-1 (308, 124, 114, 98, 62, and 42 p.p.b.) were well above the established guideline of 20 p.p.b.

Moisture content of positive samples when graded ranged from 9.4 to 20.6% (Fig. 1). Obviously the moisture level (9.4%) in F-4559 that contained 114 p.p.b. aflatoxin B-1 had not been that low during toxin formation. The three samples with 124, 308, and 141 p.p.b. B-1 were at moisture levels (19.1, 20.5, and 20.6%) favorable for toxin formation at the time they were graded. Other

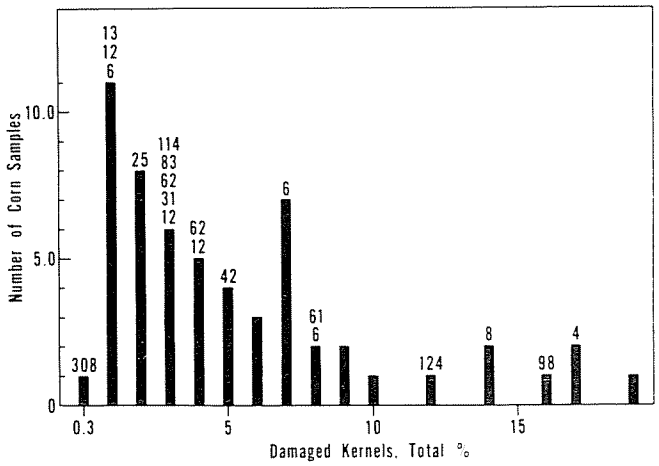

Fig. 3. Percentage of damaged kernels in southern corn samples assayed for the presence of aflatoxin. Numbers indicate levels of aflatoxin (p.p.b.) found in a positive sample with that percent damaged kernels.

samples containing more than 16% moisture would have supported mold growth. Moisture levels of corn when graded are not too significant because they do not indicate the history of the corn, including the characteristics of lots of corn that were combined during collection and marketing. The percentage of broken corn and foreign material along with the levels of aflatoxin in positive samples is shown in Fig. 2. No obvious relationship exists between aflatoxin occurrence and percentage of broken corn and foreign material. Inspection of Table III and Fig. 3 reveals that over half of the southern corn samples containing aflatoxin had less than 5% damaged kernels. Practically all positive samples from previous surveys contained more than 5% damaged kernels and, in these surveys, a relation between a high percentage of damaged kernels and aflatoxin contamination appeared to exist.

The higher incidences of aflatoxin in yellow and white corn from the South are probably caused by the more favorable conditions for mold growth and possible aflatoxin formation. Temperatures are higher for longer periods of time facilitating aflatoxin production by *A. flavus* if moisture content of corn is not carefully controlled.

Conclusion

Sixty samples from all grades except U.S. No. 1 were collected by grain inspectors of Agricultural Marketing Service from areas in the South and assayed for aflatoxin. Corn from these areas accounts for only 4% of the total yearly production in the U.S. None of 12 samples of the 1969 crop collected in the Southeast contained aflatoxin, but 14 of 22 samples of the 1970 crop in the same area were aflatoxin positive. Corn in this area was infected with southern corn leaf blight in 1970, but there is insufficient evidence to establish a definite relation between blight and aflatoxin occurrence. The 60 samples included both white and yellow corn. Four of the 11 white corn samples assayed contained aflatoxins in levels from 6 to 141 p.p.b. B-1. Two of these samples had more than 20 p.p.b. B-1, the guideline level (141, 42 p.p.b. B-1). The positive samples were in U.S. grade Nos. 4 and 5, with the highest levels in U.S. grade No. 5. Seventeen of the 49 yellow corn samples analyzed contained aflatoxins (4 to 308 p.p.b. B-1). Ten of these had more than 20 p.p.b. B-1 (308, 124, 114, 98, 83, 61, 62, 62, 31, 25 p.p.b. B-1). The yellow corn samples with the highest levels of aflatoxin were in the poorest grades. *Aspergillus flavus* was easily isolated from all positive samples. As a result of this study, the FDA intensified its program on monitoring corn for aflatoxin. Another outcome of the study was the initiation of two surveys of corn and corn meal by the Grain Division, Agricultural Marketing Service.

Acknowledgments

We thank Clemmer B. Marcus, Grain Division, Agricultural Research Service, for assistance in collecting the samples used in this study, and Mrs. Anna M. Jepson for preparation of samples for extraction.

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[Received December 12, 1972. Accepted January 25, 1973]

Reprinted from CEREAL SCIENCE TODAY July 1973, Volume 18, No. 7
Published by the American Association of Cereal Chemists, Inc.
3340 Pilot Knob Road, St. Paul, Minnesota 55121